A NEW GAS METABOLISM APPARATUS FOR SMALL LABORATORY ANIMALS

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FIVE FIGURES

Many studies on gas metabolism apparatus for small laboratory animals have been reported (Belaak and Illenyi, 1935; Junkmann, 1935; Tanaka, 1938; Harne and Teitelbaum, 1940; van Winkle, 1941; Sato, 1953), but their close examination reveals some difficult problems, *e.g.* (1) how to maintain normal physiological conditions of environment for test animals during observations; (2) how to observe both carbon dioxide and oxygen gas exchange at any desired interval, and (3) how to record automatically the exchange of componental gases. A new apparatus was devised and its conditions were studied from physiological and physico-chemical view point.

Principle: Carbon dioxide produced by a test animal in a closed animal chamber is absorbed by 25% potassium hydroxide solution and with keeping the animal under a definite physiological conditions for a definite period the volume of oxygen consumed during that period is pressured manometrically into the animal chamber. At the same time, a constant volume of potassium hydroxide solution is taken out for the determination of the carbon dioxide produced. Oxygen consumed may be recorded by the microrecorder in the high-frequency bridge circuit.

Description of the apparatus: Figure 1. shows the schema of the apparatus. A test animal in the cage (B) is placed into the animal chamber (A). In order to absorb the produced carbon dioxide quantitatively, air in the animal chamber is forced to circulate through the alkaline layer (C) by gear pump (D). The flowmeter (E) and snake tube (F) are connected with this circulating system. The pressure change in the chamber is read by manometer (M_1), and also this manometer is plated with silver on the surface of the manometer tube and served as one of the condensers of the high-frequency bridge circuit for the automatic recording of the consumed oxygen as shown in Figure 2. At any desired interval, oxygen is newly supplied manometrically by the manometer (M_1) and (M_2) through the trap (I) from the oxygen reservoir (H). The outlet of alkaline solution (J), air inlet tube (K) and thermometer (G) are also equipped to the animal chamber.

The detail of the high-frequency bridge circuit in the automatic recording is shown in Fig. 2.

Procedure: A definite volume of 25% potassium hydroxide solution is measured

Fig. 1. Schema of gas metabolism apparatus.

- A: 15L. of desiccator for animal chamber.
- B: Cage with liquid paraffine layer at the bottom so as to prevent the effect of excretion of animal.
- C: 25% potassium hydroxide solution for absorption of the carbon dioxide produced.
- D: Gear pump operating in high viscosity oil. During observation, air in the animal chamber is forced to circulate through alkaline layer by this gear pump.
- E: Flowmeter for a circulating air current of animal chamber.
- F: Snake tube.
- G: Thermometer.
- H: Oxygen reservoir.
- I: Trap with liquid paraffine.
- J: Outlet for alkali to determine the carbon dioxide produced.
- K: Air inlet tube filled with potassium hydroxide pellets.
- M₁: Manometer for the pressure change of animal chamber and also one of the four condensers of the high-frequency bridge for the automatic recording of consumed oxygen. The manometer fluid is N sulphuric acid.
- M₂: Manometer for the pressure of the oxygen reservoir.

accurately and all systems are prepared as in Figure 1. Oxygen reservoir is filled with pure oxygen from the oxygen bomb and inner pressure of the reservoir is adjusted to the outer atmospheric one. The water-level in the reservoir at this time is recorded as the initial level. After the conditions of the system become equivalent, the observation starts. Stop-cock 3, 7, and 8 are closed. At first, 25% potassium hydroxide solution is taken out for determination of initial carbon dioxide. Then the system is enclosed with cock 6. Gradually the inner pressure of the animal chamber is decreased and its change in pressure reduction is checked by the manometer M_1 . After any desired interval, oxygen is pressured into the chamber from the oxygen reservoir by opening the cock 3 until the level of the

Fig. 2. High-frequency bridge circuit for automatic recording.

manometer falls to the initial level, considering the barometric pressure change during that time. Then the three-way cock 1 is turned to the manometer M_2 and the manometer level is corrected to the initial level. The rise of the water-level in the oxygen reservoir is equivalent to the oxygen consumed in that interval. Just after the oxygen supply, the alkaline solution is taken out for titration of carbon dioxide, introducing air through cock 6.

The automatic recording of consumed oxygen is achieved by connecting the circuit at the start of the observation.

Discussion: Carbon dioxide produced was determined by titration in 25% potassium hydroxide solution with *N* sulphuric acid by the Warder's method. The air of the animal chamber was circulated at the velocity of 1.5L. per minute with the gear pump. This rate of gas flow caused at least three times circulations through the alkaline solution, hence the absorption of carbon dioxide produced in the system proceeds quantitatively.

The partial pressure of carbon dioxide in the system is less than 0.07%. The rate of pressure reduction in the chamber is about 3~5mm. Hg per 30 minutes. Because of the large capacity of the animal chamber the ratio of nitrogen and oxygen content in its atmosphere is about 78 to 19.8 within 30 minutes as compared with supposed ratio of 78 to 20 in normal air. Under these partial gas pressures, physiological conditions of the animal may be regarded as almost normal.

In the present series of experiments, the humidity is saturated, but on the surface of 25% potassium hydroxide solution the saturated vapour pressure is about 10mm. Hg at 20°C., as compared with that of 17.5mm. Hg on the water. The thermal expansion of circulating air with rotatory friction heat of the gear pump was not observed. The system of the automatic recording is an applica-

Fig. 3. Effect of propylene glycol (0.5cc./100g. intraperitoneally) upon the gas metabolism in rat.
 rat: female, 81g. (17°C, 760 mm. Hg.)
 ordinate: volume of consumed oxygen and produced carbon dioxide (cc./hr.).
 abscissa: time (hour).
 N, T: oxygen consumption of normal and treated rat, respectively.
 N', T': carbon dioxide production of normal and treated rat, respectively.

Fig. 4. Effect of DL thyroxine (8mg./100g. subcutaneously) upon the oxygen consumption in rat.
 rat: male, 160g. (17°C, 760 mm. Hg.)
 ordinate: volume of consumed oxygen (cc./g. min.).
 abscissa: time (day).

Fig. 5. Automatic recorded graph of oxygen consumption in normal rat.
 rat: female, 95g. (17°C, 760 mm. Hg.)
 longitude: electric current (μ A.) in the high-frequency bridge and it is proportional to consumed oxygen.
 abscissa: time (hour).

tion of the previously reported high-frequency bridge circuit (Ishidate *et al.*, 1954.).

This graphing method could probably be applied generally instead of the usual kymography. The examples of the results obtained with the apparatus described are shown in Figs. 3, 4, and 5.

SUMMARY

- 1) An apparatus for the study of gas metabolism in small laboratory animals has been described. This apparatus has the advantages of keeping normal physiological conditions at least several hours to the test animal and determining of both oxygen consumed and carbon dioxide at any desired interval.
- 2) The new method of automatic recording with the high-frequency bridge circuit is suggested.
- 3) An example of the results obtained with the apparatus is also shown.

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